Bioavailability and extraction of heavy metals from contaminated soil by *Atriplex halimus*

Javier Pérez-Esteban , Consuelo Escolástico , Juan Ruiz-Fernández , Alberto Masaguer , Ana Moliner

A B S T R A C T

Pot experiments were performed to evaluate the phytoremediation capacity of plants of *Atriplex halimus* grown in contaminated mine soils and to investigate the effects of organic amendments on the metal bioavailability and uptake of these metals by plants. Soil samples collected from abandoned mine sites north of Madrid (Spain) were mixed with 0, 30 and 60 Mg ha^{-1} of two organic amendments, with different pH and nutrients content: pine-bark compost and horse- and sheep-manure compost. The increasing soil organic matter content and pH by the application of manure amendment reduced metal bioavailability in soil stabilising them. The proportion of Cu in the most bioavailable fractions (sum of the water-soluble, exchangeable, acid-soluble and Fe–Mn oxides fractions) decreased with the addition of 60 Mg ha^{-1} of manure from 62% to 52% in one of the soils studied and from 50% to 30% in the other. This amendment also reduced Zn proportion in water-soluble and exchangeable fractions from 17% to 13% in one of the soils. Manure decreased metal concentrations in shoots of *A. halimus*, from 97 to 35 mg kg^{-1} of Cu, from 211 to 98 mg kg^{-1} of Zn and from 1.4 to 0.6 mg kg^{-1} of Cd. In these treatments there was a higher plant growth due to the lower metal toxicity and the improvement of nutrients content in soil. This higher growth resulted in a higher total metal accumulation in plant biomass and therefore in a greater amount of metals removed from soil, so manure could be useful for phytoextraction purposes. This amendment increased metal accumulation in shoots from 37 to 138 mg pot^{-1} of Cu, from 299 to 445 mg pot^{-1} of Zn and from 1.8 to 3.7 mg pot^{-1} of Cd. Pine bark amendment did not significantly alter metal availability and its uptake by plants. Plants of *A. halimus* managed to reduce total Zn concentration in one of the soils from 146 to 130 mg kg^{-1}, but its phytoextraction capacity was insufficient to remediate contaminated soils in the short-to-medium term. However, *A. halimus* could be, in combination with manure amendment, appropriate for the phytostabilization of metals in mine soils.

1. Introduction

Heavy-metal pollution of soils is a widespread problem. Human activities, such as metalliferous mining, are major sources of metal contamination in soils (Alloway, 1995; Ghosh and Singh, 2005a). Metal concentrations found in contaminated soils frequently exceed background levels or the levels required as nutrients, which results in their accumulation in plants to unacceptable levels. This accumulation can be a concern for human and animal health, decreased plant growth and ground cover, and negative impacts on soil microorganisms (McGrath et al., 1995; Vamerali et al., 2010). Moreover, mine soils often present other problems, such as high acidity, low nutrient status and poor physical structure, which limit the establishment of vegetation (Tordoff et al., 2000). Thus, the reduction of toxic metals and the improvement of soil conditions are necessary for the remediation of these soils.

Several techniques have been employed to remediate soils, but most of them are expensive and often impractical. A promising strategy for metal-contaminated sites is phytoremediation, which is defined as the use of green plants to remove pollutants from the environment or to render them harmless (Cunningham and Berti, 1993; Vangronsveld et al., 2009). Several specific subsets of metal phytoremediation are being developed (Kidd et al., 2009). One of these techniques is phytoextraction, in which metal-accumulating plants and appropriate soil amendments are used to transport and concentrate metals from the soil into the aboveground shoots, which are harvested with conventional agricultural methods (Kumar et al., 1995; Koopmans et al., 2008). Phytoextraction has focused on hyperaccumulator plants, which are capable of accumulating extremely high concentrations of metals in their aerial biomass without being injured (McGrath and Zhao, 2003; Dickinson et al., 2009). However, hyperaccumulator plants have some disadvantages: they often possess small biomass, they are usually only tolerant to one or two metals, and they are sensitive to climate conditions. Other phytoremediation technique is phytostabilization, a process wherein plants are used to reduce the bioavailability of metals in soil by accumulating them into the roots or by their precipitation in the rhizosphere (Mendez et al., 2007; Salt et al., 1995).

The ideal plant species for remediating a metal-contaminated soil should be fast growing, deep rooted, easily propagated and capable of tolerate and accumulate the contaminants of interest (Ebbs and Kochian, 1997; Ghosh and Singh, 2005b). Species belonging to the genus *Atriplex* are especially interesting because of their high biomass production associated with a deep root system that is able to cope with the poor structure and xeric characteristics of several polluted substrates. *Atriplex* species are not hyperaccumulators but they can tolerate and accumulate metals (Lefèvre et al., 2009; Lutts et al., 2004). Mediterranean saltbush (*Atriplex halimus*) is a halophytic species that is present as a natural invading shrub in several mining areas of northern Africa and southern Europe.

The high concentrations of labile metals in mine soils could be phytotoxic for non-hyperaccumulator plants, and the poor conditions of these soils could lead to low plant yields. The incorporation of organic amendments, such as manure compost, can improve the fertility and physical and chemical properties of the soil, thereby allowing plant survival and growth. These amendments can also reduce the bioavailability of heavy metals in soil but increase the accumulation of metals in plant shoots because of the increasing biomass (Lin et al., 2009). The reduction of metal bioavailability is due to the ability of organic compounds to redistribute metals from soluble and exchangeable forms to fractions associated with organic matter, carbonates or Fe–Mn oxides. The reduction of metal bioavailability also depends on the metal and type of soil involved, the degree of humification of the organic matter, the concentrations of heavy metals and salts, and the effects of the metals on the redox potential and soil pH (Clemente et al., 2005; Narwal and Singh, 1998; Shuman, 1999; Walker et al., 2004).

The aim of this study was to investigate the effects of two organic amendments (pine-bark compost and manure compost), with different pH and nutrients content, on the metal concentrations in plants and the fractionation and bioavailability of metals in contaminated acid mine soils. A pot experiment was carried out with plants of *A. halimus* to evaluate its growth, tolerance, metal accumulation and translocation to aerial biomass in relation to the chemical forms of metals and soil conditions.

2. Material and methods

2.1. Soil samples and organic amendments

Two heavy-metal-contaminated soils from north of Madrid (Spain) were selected for this study. The first site ("Garganta") is situated at the village Garganta de los Montes, which is close to an abandoned copper mine. The second site ("Cuadron") is situated in El Cuadron, where an old blend mine is located. Soil samples were collected from the top 20 cm. The samples were air-dried and sieved to <2 mm for analysis. The main properties of these soils are shown in Table 1. Both samples were slightly acidic, loamy sand, and poor in organic matter; they also contained high concentrations of Fe and Mn oxides and presented low salinity and cation exchange capacity (CEC). Garganta soil contained total concentrations of Cu and Cd that exceeded the European Union maximum permitted levels for agricultural soils that receive sewage sludge at pH 6–7 (Council of the European Communities, 1986). The Cuadron soil's total concentration of Cu also exceeded these limits.

Two organic amendments were added to these soils: a compost made of sheep and horse manure ("manure"), and a compost of pine bark, wood fiber and peat ("pine bark"). Some properties of these amendments are also shown in Table 1. Total metal concentrations were very low in the pine bark amendment. However, the total Zn concentration of manure was similar to the concentration observed in Garganta soil and it was higher than the Zn concentration of Cuadron soil. Thus, the application of manure cannot help to reduce the Zn concentration of soils with a low metal content.

Ten different treatments were prepared with mixtures of each soil and one of the amendments. The applied doses were 0, 30 and 60 Mg ha^{-1} of dry organic matter. Soils were mixed with 29.7 g (30 Mg ha^{-1}) and 59.5 g (60 Mg ha^{-1}) of manure per kg of soil, and with 15.1 g (30 Mg ha^{-1}) and 30.1 g (60 Mg ha^{-1}) of pine bark per kg of soil. Thus, treatments with Garganta soil were the following: non-amended soil (G0), soil with 30 Mg ha^{-1} of manure compost (G30 M), soil with 60 Mg ha^{-1} of manure (G60 M), soil with 30 Mg ha^{-1} of pine bark compost (G30P) and soil with 60 Mg ha^{-1} of manure (G60P). Treatments with Cuadron soils followed the same sequence: C0, C30M, C60M, C30P and C60P. All mixtures were well homogenised using a cement mixer.

Different properties were determined in the soils, amendments and prepared mixtures: pH and electrical conductivity (EC) in deionised water (1:2.5 w/w for soils and mixtures; 1:5 v/v for amendments), total organic matter (TOM) by the loss-on-ignition method (16 h at 400 °C for soils and 6 h at 450 °C for amendments), oxidisable organic carbon (OXC) by the modified Walkley–Black procedure described by Nelson and Sommers (1996), CEC by the barium chloride method (Rhoades, 1982), exchangeable cations (Ca, Mg and K) extracted by barium chloride, Fe and Mn oxides extracted by an ascorbate–oxalate method (Shuman, 1982), and texture by the Bouyoucos hydrometer method (Day, 1965). All analyses were performed in triplicate and the values were adjusted for oven-dried soil. The characterization of the prepared treatments is shown in Table 2.

2.2. Pot experiments

For the pot experiment, plants of *A. halimus* were used. Plants were grown in 0.7 L terracotta-colored polyethylene pots filled with 700 g of the soil and amendment mixtures described in Section 2.1. The bases of the pots were covered with a PVC coated fiberglass mesh and with a 2–3 cm layer of gravel. Pots were placed in a greenhouse with four replicates per treatment and disposed in a quincunx arrangement with 30 cm between pots. A total of 40 pots were prepared.

Seedlings of *A. halimus* purchased from a commercial nursery were prepared in root boxes with perlite and environmental control for 44 d. After their roots were washed and pruned, one seedling was transplanted into each pot. Plants were harvested 393 d after transplanting (April 2008–May 2009).

Plants were watered with a nutrient solution containing 0.17 g L^{-1} $Ca(NO_3)_2$; 0.50 g L^{-1} KNO_3; 0.16 g L^{-1} $H_2PO_4NH_4$; and 0.20 g L^{-1} NH_4NO_3 (pH 4.1; EC = 1.23 dS m^{-1}). This solution was added manually every one or two days (30–60 mL) to keep the water content near field capacity while avoiding leaching. Temperature and insolation were also controlled in the greenhouse.

After the harvest, the plants were cut at ground level. The roots were rinsed with deionised water, and the roots and aerial organs were dried in an oven at 65 °C for 48 h. The shoots and root dry weight was measured and the plant material was ground for metal determinations.

Table 1
Properties of the initial soils and organic amendments.

Characteristic	Garganta	Cuadron	Manure	Pine bark
Clay (%)	3.1	3.3	nd[c]	nd
Silt (%)	18.8	17.3	nd	nd
Sand (%)	78.2	79.4	nd	nd
pH	6.2	5.5	9.4	5.7
EC (dS m^{-1})	0.08	0.10	4.95	0.40
CEC ($cmol_c$ kg^{-1})	4.74	4.79	nd	nd
TOM (%)[a]	1.52	2.39	47.0	79.0
OXC (%)[a]	0.74	1.37	16.9	41.5
Fe oxides (g kg^{-1})	11.9	7.31	3.82	2.27
Mn oxides (g kg^{-1})	0.26	0.28	0.38	0.10
Total Cu (mg kg^{-1})[b]	913	248	30.9	1.07
Total Zn (mg kg^{-1})	203	146	179	35.7
Total Cd (mg kg^{-1})	3.68	1.32	<dl[d]	<dl

[a] TOM: total organic matter; OXC: oxidisable organic carbon.
[b] European Union limits (mg kg^{-1}) for agricultural soils receiving sewage sludge (pH 6–7): Cu 50–140, Zn 150–300, Cd 1–3 (Council of the European Communities, 1986).
[c] nd, not determined.
[d] <dl, below detection limits (Cd < 0.02 mg L^{-1} in the extract).

Soil samples collected from the pots after the harvest of the plants were homogenised, air-dried and analysed for pH, EC (H_2O 1:2.5 w/w) and metal fractionation.

2.3. *Determination of heavy metals in plants and metal fractionation in soils*

Total heavy-metal contents (Cu, Zn, and Cd) in the soil samples were determined using microwave-assisted acid digestion of the soils with *aqua regia* (ISO 11466, 1995). One gram of air-dried ground sample was placed in a Teflon vessel with 2.35 mL HNO_3 65% and 7 mL HCl 37%. The vessel was placed in the microwave at 190 °C for 25 min. After the vessel was cooled, the solution was filtered and diluted to 50 mL with deionised water.

Organic amendments were digested by weighing out a 0.25 g sample and placing it in a vessel with 2 mL HNO_3 65%, 6 mL HCl 37% and 1 mL H_2O_2 30%. The microwave was programmed to maintain the temperature at 190 °C for 25 min. The solution was filtered after cooling and diluted to 50 mL.

For plant samples, the digestion of 0.2–0.5 g of ground dry matter (roots and shoots) was accomplished by a dry ashing procedure (Tüzen, 2003) at 450 °C for 4 h. The ashes were subsequently dissolved in 5 mL HNO_3 (25% v/v). The solutions were filtered and diluted to 10 mL.

The metal fractionation of soil samples after plant harvest was carried out following the sequential extraction procedure of Tessier et al. (1979) as described in Ma and Rao (1997), with the exception of the residual fraction, which was extracted by HNO_3–HCl acid digestion. This procedure is designed to separate metals into six operationally defined fractions: water-soluble, exchangeable, acid-soluble, bound-to-Fe–Mn oxides, bound-to-organic-matter-and-sulphides and residual.

Metal concentrations in the soil and plant extracts were determined by atomic absorption spectrophotometry (AAS).

All reagents used were analytical grade or better. Doubly deionised water was used for all dilutions. All of the plastic and glassware were soaked in 5% HNO_3 overnight and rinsed with distilled water prior to use.

2.4. *Statistical analyses*

Statistical treatments of the experimental data were performed using SPSS 17.0 (SPSS, Inc.) software. The normal distribution of the data was checked by the Shapiro–Wilk's test. Variables that were not normally distributed were transformed and checked again. Means were compared through one-way ANOVA using Tukey's test ($P < 0.05$). Relationships between different parameters were determined by Pearson's correlation coefficients (r) using a two-tailed test. Standard errors (SEs) were calculated to determine the variability of the means between replicates.

3. Results and discussion

3.1. *Metal fractionation*

A sequential extraction procedure was carried out in soil samples after the harvest of plants to study the effect of organic amendments on the chemical fractionation of the metals. Fig. 1 shows Cu and Zn distributions among the different operationally defined fractions in each treatment and the total concentration of

Table 2
Properties of soils and mixtures with organic amendments before the harvest of plants.

Treatment	pH	EC (dS m^{-1})	OXC (%)	CEC ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})
G0	6.2b ± 0.1	0.08a ± 0.01	0.74a ± 0.08	4.74a ± 0.05	0.50a ± 0.00	0.48a ± 0.07	1.19a ± 0.00
G30M	6.5c ± 0.1	0.51c ± 0.03	1.17bc ± 0.02	5.28ab ± 0.10	0.76b ± 0.03	2.31b ± 0.06	1.40b ± 0.08
G60M	6.8d ± 0.0	0.94d ± 0.01	1.33c ± 0.11	5.99b ± 0.20	1.00c ± 0.07	4.38c ± 0.17	1.76c ± 0.11
G30P	5.9a ± 0.0	0.11ab ± 0.00	0.88ab ± 0.06	5.30ab ± 0.12	0.59ab ± 0.03	0.55ab ± 0.05	1.31a ± 0.02
G60P	5.6a ± 0.0	0.17b ± 0.00	1.16bc ± 0.10	5.68ab ± 0.42	0.63ab ± 0.01	0.59ab ± 0.04	1.34a ± 0.01
C0	5.5b ± 0.0	0.10a ± 0.00	1.37a ± 0.02	4.79a ± 0.10	0.61a ± 0.01	3.28a ± 0.04	0.66a ± 0.02
C30M	5.8c ± 0.0	0.58c ± 0.03	1.75c ± 0.01	5.67ab ± 0.21	0.93ab ± 0.08	4.00b ± 0.21	2.00b ± 0.08
C60M	6.1d ± 0.0	0.99d ± 0.04	1.96d ± 0.05	6.16b ± 0.11	1.09b ± 0.04	4.09b ± 0.14	3.08c ± 0.07
C30P	5.4a ± 0.0	0.16b ± 0.00	1.57b ± 0.05	4.87a ± 0.11	0.64a ± 0.02	3.16a ± 0.07	0.72a ± 0.02
C60P	5.3a ± 0.0	0.16b ± 0.00	1.74c ± 0.00	5.89ab ± 0.66	0.64a ± 0.03	3.38a ± 0.07	0.74a ± 0.00

G0: non-amended Garganta soil; G30M and G60M: Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; G30P and G60P: Garganta with 30 and 60 Mg ha^{-1} of pine bark; C0, C30M, C60M, C30P and C60P: the same sequence for Cuadron soil.
Mean ± SE, $n = 3$.
Values within a column followed by the same letter are not significantly different among treatments of the same soil.

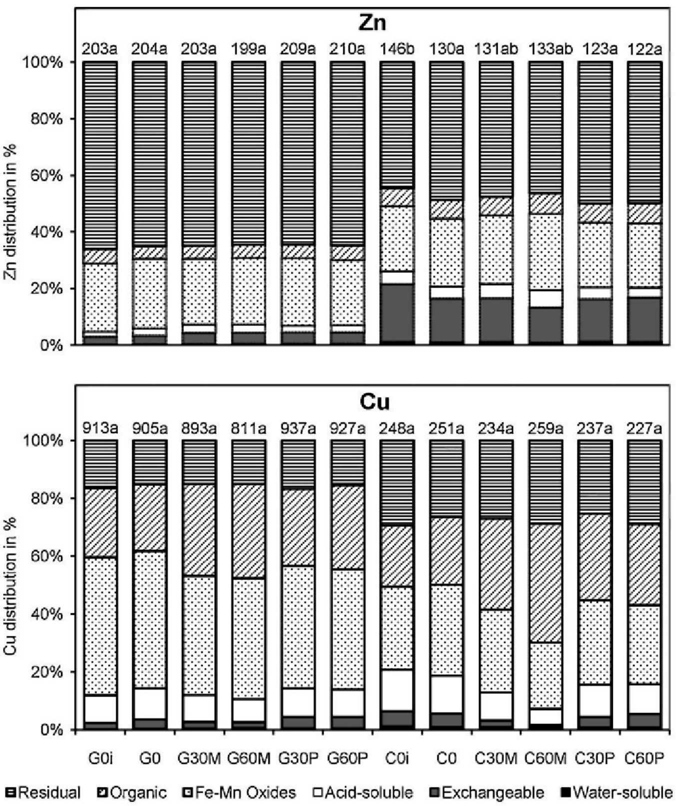

Fig. 1. Percentages of Zn and Cu fractions in soil with respect to the sum of all fractions at the beginning of the experiment in non-amended soils and at the end of the experiment in the different treatments, obtained by a sequential extraction procedure (Tessier et al., 1979). G0: non-amended Garganta soil; G30M and G60M: Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; G30P and G60P: Garganta with 30 and 60 Mg ha^{-1} of pine bark; C0, C30M, C60M, C30P and C60P: the same sequence for Cuadron soil. G0i and C0i: non-amended soils at the beginning of the experiment. Column fractions represent the mean of four replicates. Values above columns represent total metal concentration in mg kg^{-1} extracted by digestion with *aqua regia*. Values with the same letter are not significantly different among treatments of the same soil.

these metals extracted by digestion with *aqua regia*. In almost every case, the Cd concentration was below the detection limit of the instrument (<0.02 mg L^{-1} in the extract); it is therefore not shown in the figure.

In both soils, Cu was found mostly in the less-labile fractions, although a small proportion of Cu was associated with the water-soluble and exchange fractions. These results indicate a low Cu bioavailability in the studied soils. Although Cu forms strong complexes with organic ligands, the highest amount of Cu in Garganta soil was found in the Fe–Mn oxides fraction, following the order: Fe–Mn oxides > organic > residual > acid-soluble > exchangeable > water soluble. Other authors (Narwal and Singh, 1998) have reported that the largest fraction of Cu was associated with the organic compounds. However, the large amount of Fe and Mn oxides and the low organic matter content in Garganta soil resulted in a greater proportion of Cu bound to Fe–Mn oxides. In the Cuadron samples, the residual, organic-bound and Fe–Mn oxides fractions of Cu presented similar proportions, whereas the acid-soluble, exchangeable and water-soluble fractions were of minor importance. The Cuadron soil presented a lower total Cu concentration than the Garganta soil.

Zn was mostly concentrated in the residual fraction in both soils, but a significant amount of this metal was also associated with the Fe–Mn oxides fraction, which agrees with the results of previous reports (Li and Thornton, 2001; Ma and Rao, 1997; Narwal and Singh, 1998). The other fractions presented lower proportions. Despite its lower total Zn concentration, the proportion of exchangeable Zn in Cuadron was relatively high and greater than that in Garganta, probably because of the lower pH and Fe–Mn oxides content of the Cuadron soil.

The addition of manure compost to both soils significantly decreased the proportion of Cu in the exchangeable, acid-soluble and Fe–Mn oxides fractions ($P < 0.05$), which reduced the bioavailability of this element. This loss in the more-labile fractions was balanced by a significant increase in the organic-bound Cu ($P < 0.05$). The application of pine bark also raised the organic-bound Cu proportion ($P < 0.05$), and this increase was mainly associated with a significant decrease in the acid-soluble and Fe–Mn oxides fractions ($P < 0.05$) and not with the exchangeable fraction. The high pH of the manure amendment and the contribution of organic matter by both amendments affected the adsorption sites of these variable-charge soils and therefore changed the Cu distribution in the soils. The residual fraction was not significantly affected by the application of organic amendments.

No differences were observed in the Zn distribution between the different treatments in the Garganta samples, but the addition of a high dose of manure amendment in the Cuadron soil significantly decreased the Zn proportion in the exchangeable fraction ($P < 0.05$) and increased the amount of Zn in the acid-soluble and Fe–Mn oxides fractions ($P < 0.05$). This reduction of Zn availability was due to the high pH of this amendment, which raised the surface charges of Fe and Mn oxides. The application of organic materials did not result in a higher amount of Zn associated with the organic fraction, unlike Cu. Similar results were obtained by Shuman (1999), who observed that the addition of organic materials with high pH redistributed Zn from the exchangeable fraction to the Fe–Mn oxides fraction. Xian and Shokohifard (1989) also found that the proportion of Zn in the exchangeable fraction increased and that Zn in the acid-soluble fraction decreased when soil pH was lowered.

Fig. 1 also shows the differences in Cu and Zn fractions and total metal concentrations between the non-amended soils at the beginning (G0i and C0i) and at the end (G0 and C0) of the experiment. Neither the total Cu concentration nor the different Cu fractions were significantly affected by plants. However, the total Zn concentration was significantly reduced at the end compared to the beginning of the experiment in the samples of Cuadron soil ($P < 0.05$). This Zn uptake by plants was mainly obtained from the exchangeable fraction. There was a significant decrease of this fraction ($P < 0.05$), from 26 mg kg^{-1} (± 1.0) in C0i to 19 mg kg^{-1} (± 1.3) in C0.

3.2. *Plant growth*

Plant growth presented different values in the applied treatments, as indicated by the shoot and root dry weights (Fig. 2). The aerial biomass of these plants was significantly higher in treatments with manure amendment than those with pine-bark or non-amended soils. Furthermore, an increased dose of manure significantly raised the shoots weight in Cuadron soil. Root dry weight did not present significant differences, except in samples G30M and G30P.

This increased yield after manure application could be due to a lower metal bioavailability and therefore a lower metal stress. Although metal availability was higher in pine-bark-amended and non-amended soils, no chlorosis symptoms were observed in the grown plants. Significant and negative correlations were found between shoot dry weight of *A. halimus* and the sum of the first three Cu fractions (water-soluble, exchangeable and acid-soluble) in treated Garganta ($r = -0.66$, $P < 0.01$, $n = 20$) and in treated Cuadron ($r = -0.90$, $P < 0.01$, $n = 19$). However, this species was not strongly

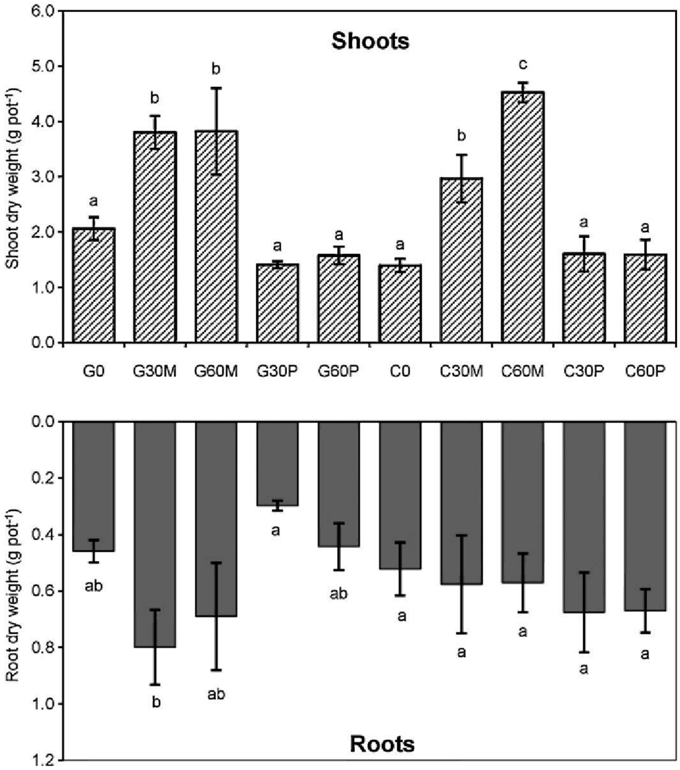

Fig. 2. Shoot and root dry weight of *A. halimus* at the end of the experiment in the different treatments. G0: non-amended Garganta soil; G30M and G60M: Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; G30P and G60P: Garganta with 30 and 60 Mg ha^{-1} of pine bark; C0, C30M, C60M, C30P and C60P: the same sequence for Cuadron soil. Columns represent the mean of four replicates. Error bars represent SE. Values with the same letter are not significantly different among treatments of the same soil.

affected by Zn availability. No significant correlations were found between total metal concentrations and plant growth.

In addition, the improvement of soil fertility with manure amendments could have contributed to the increased biomass production. Organic matter provided by manures acts as a nutrient pool, enhances nutrient cycling, increases CEC and buffer capacity, and improves the physical properties of soil (Stewart et al., 2000). In this study, manure amendments not only provided organic matter and a more adequate pH to soil, they also supplied a higher amount of nutrients as exchangeable Mg, K and Ca than pine-bark compost (Table 2). The concentration of these elements in soil was strongly and positively correlated with shoot dry weight ($r = 0.92$ for Mg, $r = 0.93$ for K, $P < 0.01$, $r = 0.71$ for Ca, $P < 0.05$, $n = 10$). The pH, COX and CEC were not correlated with plant growth.

3.3. *Metal concentrations in plants*

Cu, Zn and Cd concentrations were measured in plant shoots and roots (Fig. 3). Plants grown in Garganta mixtures concentrated a higher amount of Cu than those grown in Cuadron treatments because of the greater Cu availability in Garganta soil. However, the higher Zn availability in Cuadron soil led to a higher concentration of this metal in the tissues of plants. Cd concentration in roots was higher in Garganta, whereas Cd shoot concentration was higher in Cuadron.

The metal concentrations in plant shoots varied in the same manner as metal bioavailability in the soil. Shoot Cu concentrations in plants grown in Garganta soil significantly decreased with the addition of the manure amendment, but not with the addition of the pine-bark amendment. No significant differences were found

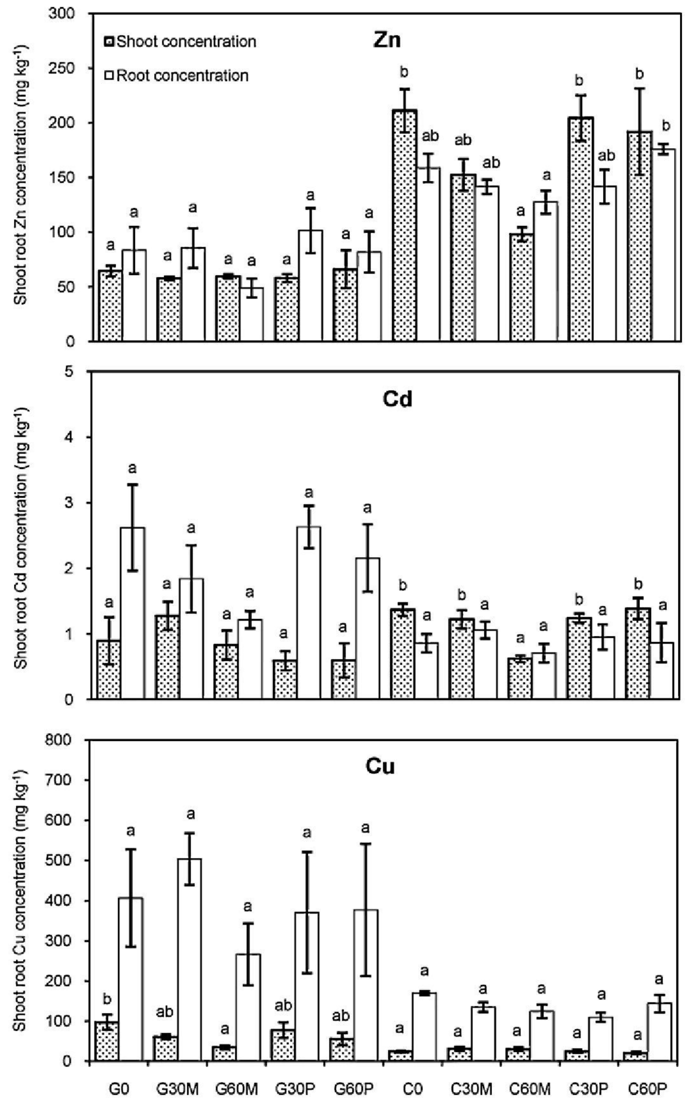

Fig. 3. Zn, Cd and Cu concentrations in shoots and roots of *A. halimus* at the end of the experiment in the different treatments. G0: non-amended Garganta soil; G30 M and G60 M: Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; G30P and G60P: Garganta with 30 and 60 Mg ha^{-1} of pine bark; C0, C30M, C60M, C30P and C60P: the same sequence for Cuadron soil. Columns represent the mean of four replicates. Error bars represent SE. Values with the same letter are not significantly different among treatments of the same soil.

among Cuadron treatments. Also, a lower shoot Zn concentration was observed when manure was added because of its high pH; the differences, however, were only significant in plants grown in Cuadron soil. Shoot Cd was also significantly lower in plants grown in C60M. No significant differences in root metal concentrations were observed among the treatments, except for Zn in plants grown in C60P.

Organic matter added by the manure amendment and its high pH reduced metal bioavailability, which affected the metal uptake by plants. There were strong and negative correlations between organic carbon, shoot and root Cu concentrations in plants ($r = -0.86$, $P < 0.01$, $n = 10$) and root Cd ($r = -0.92$, $P < 0.01$, $n = 10$). The Zn concentrations in plants were not related to soil organic matter; it was, however, negatively correlated with pH ($r = -0.90$ for shoots, $r = -0.67$ for roots, $P < 0.01$, $n = 40$). These results are in agreement with those reported by other authors (Narwal and Singh, 1998; Walker et al., 2004; Clemente et al., 2005), who observed that the addition of manure with a high pH reduced metal uptake by plants. EC and CEC were not correlated with metal concentrations in plant tissues.

A significant relationship was found between the metal concentrations in plants and the labile metal concentrations in soil. The sum of the first three Cu fractions was positively and significantly correlated with the plant Cu concentration ($r = 0.75$ for shoots, $r = 0.56$ for roots, $P < 0.01$, $n = 37$). The sum of the first three Zn fractions was also positively correlated with Zn concentration in plants ($r = 0.82$ for shoots, $r = 0.74$ for roots, $P < 0.01$, $n = 39$). The total Cu concentration in soil was also positively correlated with Cu concentration in plants ($r = 0.76$ for shoots, $r = 0.57$ for roots, $P < 0.01$, $n = 37$), but no positive correlations were observed between the total Zn and Cd concentrations in soil and the metal concentrations in plants. The measurement of total concentrations is not the best method for predicting the bioavailability of a metal in soil and its uptake by plants. Xian (1989) also found that metals in the exchangeable and acid-soluble fractions strongly controlled the uptake by plants more than the total metals content in the soil.

The amounts of metals concentrated in plant tissues did not affect plant growth. According to Kabata-Pendias and Pendias (2001), shoot metal concentrations were less than the toxic level (100 mg kg^{-1} for Cu, 400 mg kg^{-1} for Zn, and 30 mg kg^{-1} for Cd) and no chlorosis symptoms were observed. In any event, no significant correlations were found between the metal concentrations in plants and their dry weights. Other authors have reported that *A. halimus* is tolerant to Zn and Cd and that, concentrations of these metals in above-ground tissues are generally kept at low levels (Lutts et al., 2004).

The Cu concentration in roots was always higher than its concentration in the aerial organs of the plants. However, Zn and Cd concentrations in shoots were higher than the root concentrations in some treatments of Cuadron, which demonstrates a better translocation of Zn and Cd to the shoots. Lutts et al. (2004) have reported that *A. halimus* grown in a nutrient solution accumulated more Zn and Cd in its roots than in its shoots, but concentrations of these metals in shoots increased in plants exposed to Zn and Cd. The authors have suggested that *A. halimus* is a halophytic species that is capable of precipitating Cd and Zn as oxalate salts, mainly in stems, to prevent metal toxicity.

The Cu concentrations in shoots of plants grown in Garganta samples (97 mg kg^{-1}) were higher than those determined by Pastor et al. (2007) in the most abundant grassland species grown in the same soil (*Agrostis castellana*: 27 mg kg^{-1}; *Corrigiola telephiifolia*: 41 mg kg^{-1}). However, the Zn and Cd concentrations found in shoots of *A. halimus* (65 mg kg^{-1} of Zn and 0.9 mg kg^{-1} of Cd) were not higher than those reported by these authors (*Agrostis castellana*: 68 mg kg^{-1} of Zn and 1 mg kg^{-1} of Cd; *Corrigiola telephiifolia*: 61 mg kg^{-1} of Zn and 3 mg kg^{-1} of Cd). These low metal concentrations found in shoots of *A. halimus*, the low translocation of metals and the relatively high metal tolerance of this species could make it useful for the phytostabilization of metals rather than for phytoextraction. Mendez et al. (2007) found that other *Atriplex* species are good candidates for phytostabilization strategies in mine soils.

3.4. Total metal accumulation in plants

Metal concentrations in a plant should not be the only parameter used to evaluate the phytoextraction capacity of a plant because it does not take plant biomass into consideration. It is more informative to determine the total amount of metal extracted from the growing media and accumulated in the plant shoots, by calculating the product of shoot and root dry weight and their metal concentration (Fig. 4). Despite the lower concentrations of metals in plants grown in soils with manure amendments, the higher growth reached in these treatments resulted in a greater metal accumulation in the plants, especially in the aerial biomass, in comparison

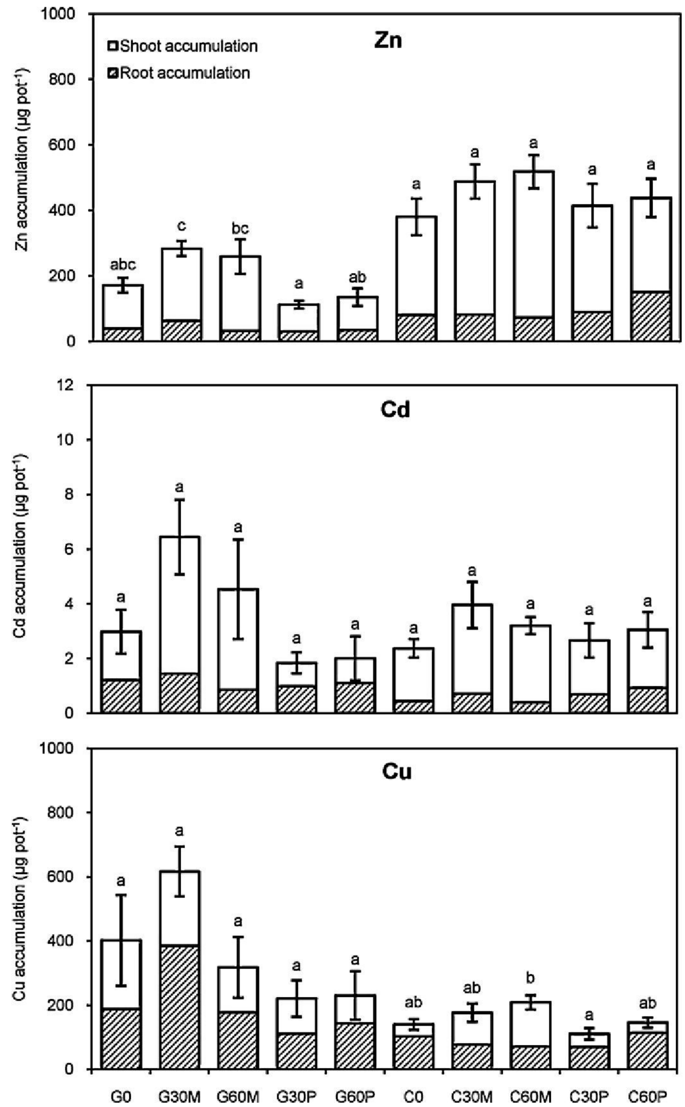

Fig. 4. Total Zn, Cd and Cu extracted per pot and accumulated in shoots and roots of *A. halimus* at the end of the experiment in the different treatments, by calculating the product of shoot and root dry weight and their metal concentration. G0: non-amended Garganta soil; G30M and G60M: Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; G30P and G60P: Garganta with 30 and 60 Mg ha^{-1} of pine bark; C0, C30M, C60M, C30P and C60P: the same sequence for Cuadron soil. Columns represent the mean of four replicates. Error bars represent SE (shoot + root). Values with the same letter are not significantly different among treatments of the same soil (shoot + root).

with pine-bark mixtures. Manure amendments could be useful not only for stabilising metals in contaminated soils, but also for achieving a higher accumulation in the harvestable parts of the plants, thereby removing metals from the soil.

Although this species reached a high biomass and a significant amount of metals accumulated in its tissues, the metal uptake was very low compared to the total metal content in the soils. To assess the feasibility of these plants for phytoextraction, the number of crop cycles required to reduce the total metal concentrations of these soils to acceptable levels was calculated as described in Clemente et al. (2005). It was considered the amount of metals to be extracted from the soil as the product of the soil dry weight in the pots and the difference between the total metal soil concentrations (Fig. 1) and the lower metal limits established by the EU for agricultural soils that receive sewage sludge at pH 6–7 (Council of the European Communities, 1986) (Cu: 50 mg kg^{-1}; Zn: 150 mg kg^{-1}; Cd: 1 mg kg^{-1}). The amount of metals to be extracted from the soil was divided by the total metal accumulation in shoots per pot (Fig. 4) to calculate the required number of crop cycles. Depending on the applied amendment, 2500–7000, 150–500, or 100–600 crop cycles of *A. halimus* would be necessary to reduce the Cu, Zn and Cd concentrations, respectively, of the Garganta soil to the maximum values permitted by the EU. For comparison, 1000–4000 crop cycles would be required to reduce Cu in Cuadron soil, which is not considered contaminated by Zn and Cd. These results make phytoextraction techniques unviable for the remediation of soils with high levels of metals in the short-to-medium term. It must be emphasised that these plants were grown in small pots, where roots may not be able to explore a significant amount of soil; field trials are therefore necessary to study the phytoextraction capacity of this species.

4. Conclusion

The chemical fractionation and distribution of metals in soil were considerably affected by the organic amendments via the alteration of soil conditions. The addition of organic matter and the high pH of manure compost significantly reduced metal bioavailability and redistributed the metals from the most labile fraction to the most stable fractions. This amendment not only stabilised metals in these contaminated soils and reduced metal toxicity, it also lowered soil acidification and provided a significant amount of nutrients for the plants, which allowed a higher biomass production. Plants grown in the manure treatments managed to remove a greater amount of metals from the soil accumulating them in their above-ground tissues due to the plants' higher growth. The application of this amendment could be a useful practice for phytoremediation techniques. In spite of its low pH, the pine-bark amendment did not significantly increase metal bioavailability. The low growth achieved in these treatments led to a decreased amount of metal extracted from the soil.

The Mediterranean saltbush was capable of developing a high biomass production without being affected by metal toxicity and managed to accumulate a significant amount of metals in shoots. However, its phytoextraction capacity was insufficient to remediate soils with high levels of metals in the short to medium term. *A. halimus* could have a better potential for phytostabilization strategies.

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